Artificial Transaminases Linking Pyridoxamine to Binding Cavities: Controlling the Geometry

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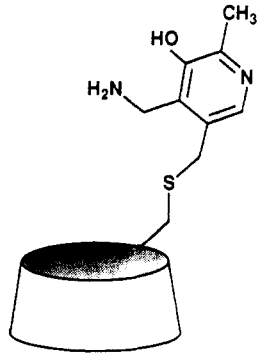
Abstract: A transaminase mimic joining a pyridoxamine unit to a cyclodextrin by a single linkage is quite selective for the conversion of phenylpyruvic acid to phenylalanine, compared with its reactivity toward nonbinding ketoacids. The selectivity is even greater with 4-*tert*-butylphenylpyruvic acid but is essentially erased with a phenylpyruvic acid carrying a *tert*-butyl group in the meta position. This strong geometric preference can be modified with transaminase mimics in which the pyridoxamine is joined to cyclodextrin by two links, greatly restricting the freedom of the system. A transaminase has also been prepared with the pyridoxamine unit doubly linked to a synthetic macrocyclic hydrophobic cavity, which has yet other geometric preferences. The doubly linked pyridoxamine units were prepared by use of a novel synthetic procedure for the conversion of olefins to *vic*-dithiols. An improved procedure for the preparation of β-cyclodextrin 6A,6B diiodide is also described.

Transaminases are a class of enzymes that synthesize amino acids from ketoacids by the simultaneous conversion of pyridoxamine phosphate to pyridoxal phosphate (Scheme I). They are normally selective for particular substrates, and they also show the stereospecificity characteristic of enzymatic processes. We have described a number of mimics of the transaminase enzymes that incorporate pyridoxamine along with other catalytically useful functions.[3,4]

In one approach, we attach pyridoxamine to a hydrophobic binding cavity so as to produce selectivity for transamination of ketoacids that can bind into the cavities. In our first example[5] we produced a compound (**1**) in which the pyridoxamine was



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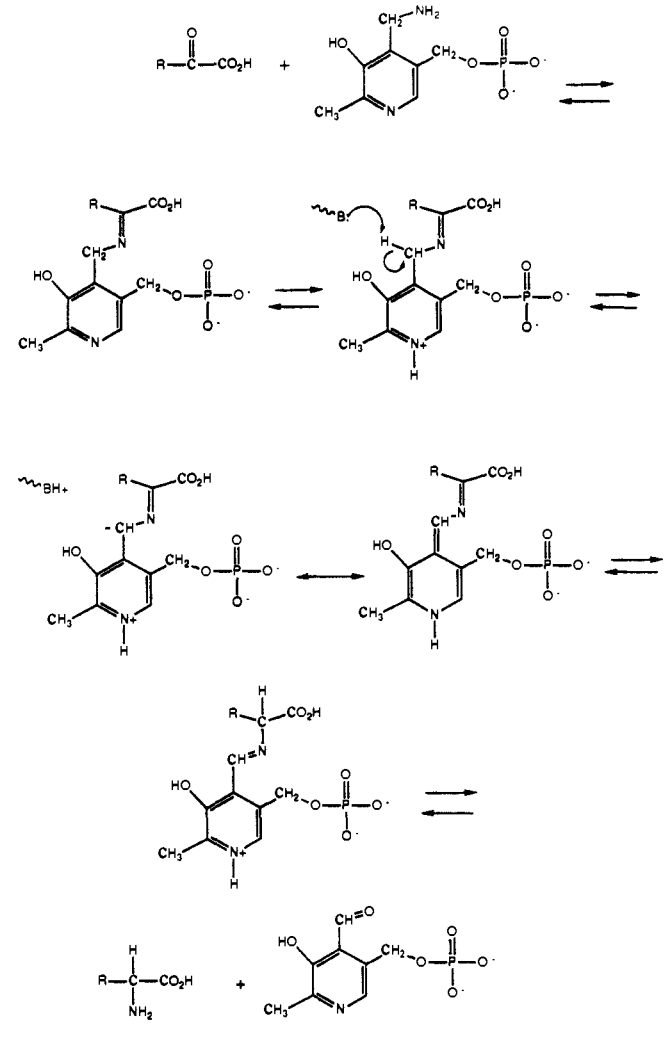
attached to the primary face of β-cyclodextrin (cycloheptaamylose) by a thioether link. This proved to be selective for the amination of phenylpyruvic acid to produce phenylalanine, but there was some ambiguity[3] about the magnitude of this preference. We have also attached pyridoxamine to the secondary face of β-cyclodextrin[6] and to a partly deoxygenated β-cyclodextrin,[7] and we have also linked it to a synthetic hydrophobic macrocycle.[8] In all cases there was preferential reaction with substrates that could bind into the cavities of these enzyme mimics.

In a different approach, we have joined pyridoxamine units to basic catalyst groups, to promote the proton transfers that are required in transamination. This led to faster rates[9,10] and to significant optical activity in the product amino acids when the basic group was asymmetrically mounted.[11] Finally, we have combined these two lines in the production of transaminase mimics that combine pyridoxamine with both a binding group and an

Scheme I. Enzymatic Transamination



(1) NIH Postdoctoral Fellow, 1988-1990.
(2) American Cancer Society Postdoctoral Feloow, 1985-1987.
(3) Breslow, R.; Czarnik, A. W.; Lauer, M.; Leppkes, R.; Winkler, J.; Zimmerman, S. *J. Am. Chem. Soc.* **1986**, *108*, 1969.
(4) Breslow, R.; Chmielewski, J.; Foley, D.; Johnson, B.; Kumabe, N.; Varney, M.; Mehra, R. *Tetrahedron* **1988**, *44*, 5515.
(5) Breslow, R.; Hammond, M.; Lauer, M. *J. Am. Chem. Soc.* **1980**, *102*, 421.
(6) Breslow, R.; Czarnik, A. W. *J. Am. Chem. Soc.* **1983**, *105*, 1390.
(7) Czarnik, A. W.; Breslow, R. *Carbohydrate Res.* **1984**, *128*, 133.
(8) Winkler, J.; Coutouli-Argyropoulou, E.; Leppkes, R.; Breslow, R. *J. Am. Chem. Soc.* **1983**, *105*, 7198.
(9) Zimmerman, S.; Czarnik, A. W.; Breslow, R. *J. Am. Chem. Soc.* **1983**, *105*, 1694.
(10) Weiner, W.; Winkler, J.; Zimmerman, S.; Czarnik, A. W.; Breslow, R. *J. Am. Chem. Soc.* **1985**, *107*, 4093.
(11) Zimmerman, S. C.; Breslow, R. *J. Am. Chem. Soc.* **1984**, *106*, 1490.

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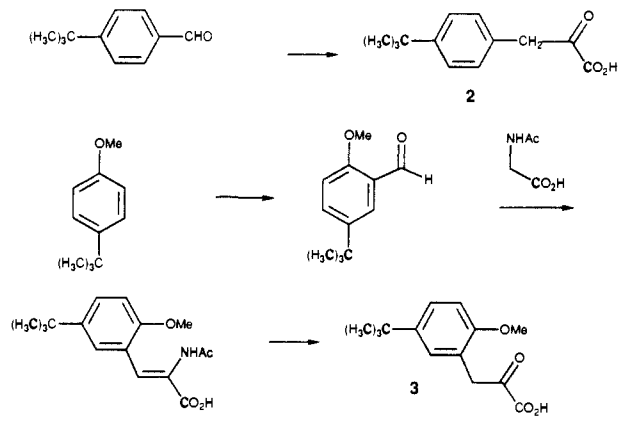
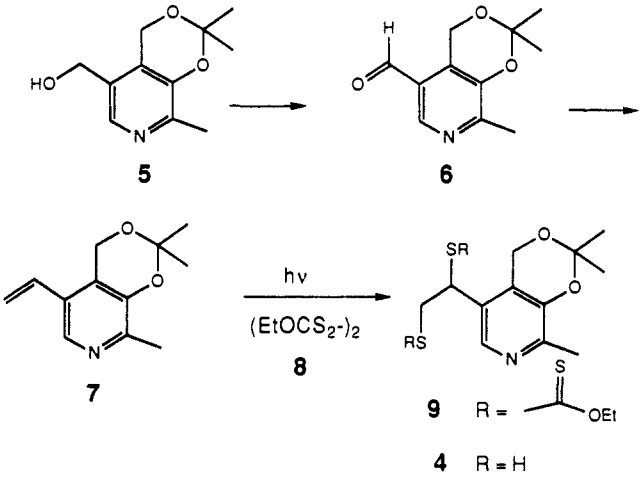
Scheme II



Scheme III



asymmetrically mounted base catalyst.[4]

We have now further explored the cooperative binding and transamination in mimics with a pyridoxamine and a hydrophobic binding unit. Careful studies establish that even in compound **1** this cooperation leads to large selectivities for substrates that can bind into the cavity. The attachment of *tert*-butyl groups to the phenylpyruvic acid substrate produces stronger binding and better defined geometry. This leads to striking differences between para- and meta-substituted derivatives.

Our previous linkages between the pyridoxamine and the binding unit involve a flexible chain with many single bonds; for example, in compound **1** there are four such single bonds. We have now devised new compounds in which some of that flexibility is frozen out. As expected, the resulting enzyme mimics show greater geometric definition, and their substrate selectivities are not the same as the more flexible **1**. In the course of this synthesis of rigid analogues of **1** and related compounds, we have devised a novel method for the conversion of an olefin to a 1,2-dithiol that may be of general use.

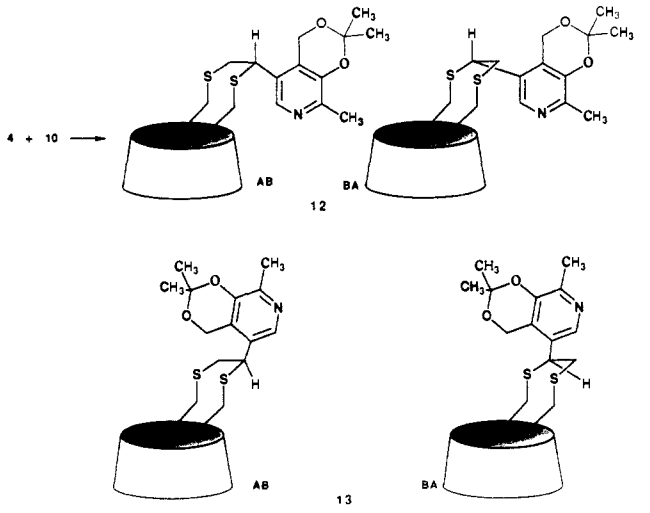
Results and Discussion

Syntheses. The new ketoacids **2** and **3** were synthesized by using the Ehrlenmeyer azlactone reaction (Scheme II).[12] *p-tert*-Butylbenzaldehyde afforded **2**, and **3** was prepared from 4-*tert*-butylanisole-2-carboxaldehyde. The methoxy group was present for synthetic convenience.

A derivative **4** of pyridoxine was prepared (Scheme III) carrying two thiol groups, so it could be doubly attached to cyclodextrin or to a synthetic macrocycle. Pyridoxine acetonide (**5**) was oxidized to the aldehyde **6**, which was converted to the ethylene derivative **7**. In the key step, this underwent free radical addition of diethyldixanthogen (**8**) to afford **9** in 80–90% yield, and with LAH this was reduced to the dithiol **4**. The air-sensitive dithiol

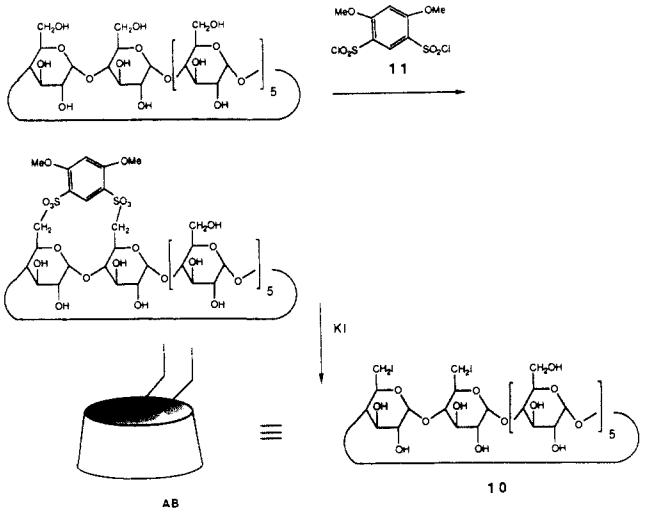
(12) Carter, H. E. *Organic Reactions* **1946**, Vol. III, Chapter 5.

Scheme IV



was directly used in the next step.

For attachment to β-cyclodextrin, we prepared the cyclodextrin 6,6′-*A*,*B*-diiodide (**10**).[13] Tabushi had reported this compound



previously,[14] prepared by reaction of the cyclodextrin with *m*-benzenebis(sulfonyl chloride) and then displacement of the two sulfonate groups with iodide ion. We found that the yield in this sequence was very low, ca. 5% based on the sulfonyl chloride even using a 12-fold excess of cyclodextrin, and the isolation was difficult. The problem is the lability of the bis-sulfonate ester, with its strongly electron-withdrawing substituents. To solve this we have used the related bis(sulfonyl chloride) **11**, carrying stabilizing methoxy groups. Now with only 1 equiv of cyclodextrin the yield of the diiodide is a workable 10–12%, with fewer decomposition products.

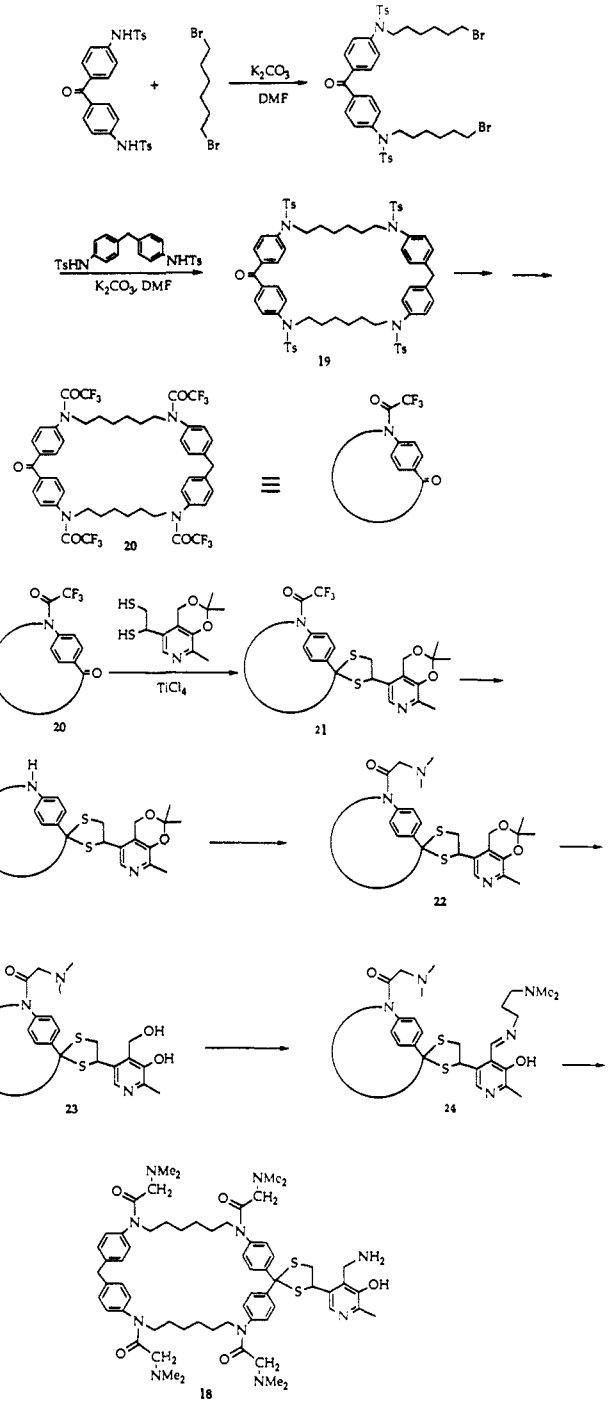
Reaction of the dithiol **4** with the diiodide **10** affords a mixture of four bis-thioethers in 25% combined yield (Scheme IV). The dithiol is a mixture of two enantiomers, and the primary thiol may be attached to either the A or the B residue of the cyclodextrin, lettering clockwise (the A and B positions are diastereotopic, because of the chirality of the glucose residues in cyclodextrin). Reverse phase chromatography separates the mixture into two pairs, one more polar and the other less polar. We assign them as the exo (**12**) and endo (**13**) isomeric pairs, respectively, based on two arguments.

The exo versus endo orientation should have a significant effect on the polarity of the compound, since it greatly changes the

(13) The glucoe residues of the cyclodextrin are labeled in clockwise sequence alphabetically, on the primary face.

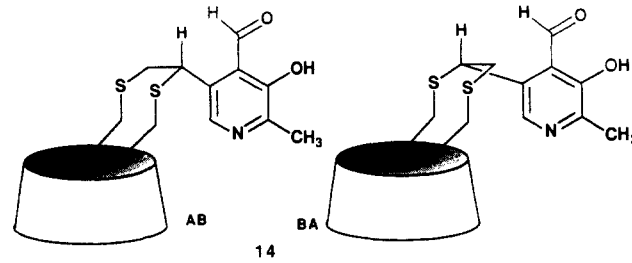
(14) Tabushi, I.; Yamamura, K.; Nabeshima, T. *J. Am. Chem. Soc.* **1984**, *106*, 5267.

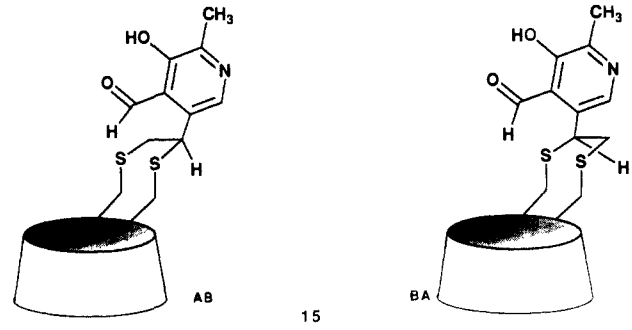
Scheme V



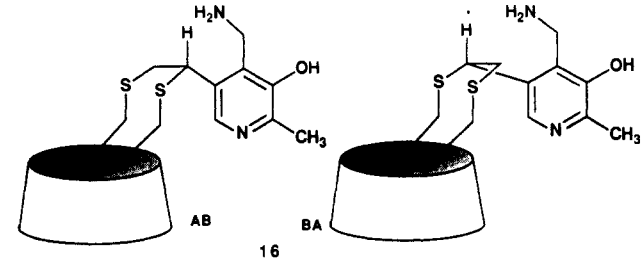
geometric relationship of the cyclodextrin to the pyridoxine appendage. However, we expect that within each geometry the difference between the A,B and B,A sequence will have small effects. They differ only by a clockwise versus counterclockwise attachment sequence, which are different only because of the glucose chirality. This seems unlikely to lead to a large difference in chromatographic behavior compared with the exo/endo difference. The second argument is that our transamination data, discussed below, show significant geometric differences between the polar and the nonpolar pairs of isomers, differences that reflect a change in the relative position of binding group and pyridine appendage in the two series. Again this seems to make the most sense for an exo/endo difference. Of course absolute proof for this assignment, and other interesting results, would come from the use of optically resolved dithiol in the synthesis.

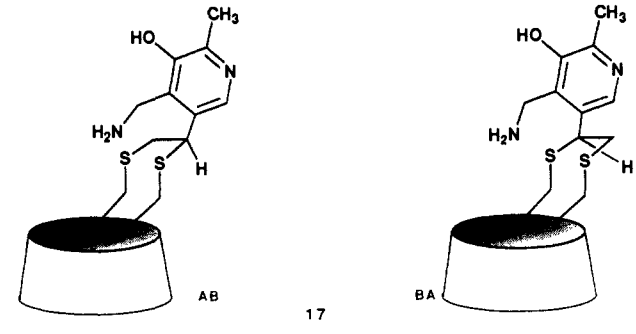
The acetonide protecting group was removed with mild acid in 90% yield without damage to the cyclodextrin, and selective oxidation with MnO_2 afforded the aldehydes **14** and **15**. These were converted to the pyridoxamine derivatives **16** and **17** by transamination with an excess of phenylyglycine.





For the synthesis of the macrocyclic derivative **18**, the sequence in Scheme V was used. The macrocyclic tetratosyl ketone **19** was converted to the tetratrifluoroacetyl derivative **20**, since the conditions needed for tosyl removal were too vigorous for later intermediates, and this ketone was then converted to the dithioketal **21** with dithiol **4**. The trifluoroacetyl groups were replaced by *N*,*N*-dimethylglycyl units in **22** to produce water solubility, and the pyridoxine was deprotected. Oxidation of **23** with MnO_2 in the presence of (dimethylamino)propylamine afforded the Schiff base **24** of the pyridoxal derivative[15] (oxidation to the aldehyde in the absence of this diamine went in very poor yield, with much additional oxidation occurring). Then the imine was transiminated with ammonia, and the product was directly reduced with $NaBH_4$ to the pyridoxamine derivative **18**.





Transaminations. Pyridoxamine and its derivatives were incubated with a ketoacid, or a ketoacid mixture for competition experiments, under the conditions indicated in the Experimental Section and in Table I. The resulting amino acids were assayed

(15) Cf.: Iwata, M. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 2835.

Table I. Selective Transaminations[a]

pyridoxamine derivative (mM)	substrate acid (mM)	observed ratios[b]	relative reactivities[c]
1. pyridoxamine (1)	α-ketoglutaric (1) phenylpyruvic (1)	Phe/Glu = 7.8 ± 0.4	Phe/Glu = 7.8 ± 0.4[d]
2. pyridoxamine (1)	α-ketoglutaric (10) phenylpyruvic (1)	Phe/Glu = 0.44 ± 0.06	Phe/Glu = 4.4 ± 0.6
3. pyridoxamine (1)	pyruvic (100) phenylpyruvic (1)	Phe/Ala = 0.015 ± 0.007	Phe/Ala = 1.5 ± 0.7
4. pyridoxamine (1)	pyruvic (2) phenylpyruvic (1)	Phe/Ala = 0.64 ± 0.03	Phe/Ala = 1.29 ± 0.06
5. pyridoxamine (0.9)	pyruvic (10) 4-*t*-BuPhepyr[e] (1)	4-*t*-BuPhe/Ala = 0.1 ± 0.01	4-*t*-BuPhe/Ala = 1.0[f]
6. pyridoxamine (0.9)	pyruvic (25) 4-*t*-BuPhepyr[e] (1)	4-*t*-BuPhe/Ala = 0.12	4-*t*-BuPhe/Ala = 3[g]
7. pyridoxamine (1)	α-ketoglutaric (5) pyruvic (2) phenylpyruvic (1)	Phe/Glu = 0.76 ± 0.1 Phe/Ala = 0.35 ± 0.05 Glu/Ala = 0.46 ± 0.1	Phe/Glu = 3.8 ± 0.5 Phe/Ala = 0.7 ± 0.1 Glu/Ala = 0.18 ± 0.1
8. compd **1** (0.7)	α-ketoglutaric (100) phenylpyruvic (1)	Phe/Glu = 7.5 ± 0.9	Phe/Glu = 750 ± 90
9. compd **1** (0.7)	pyruvic (100) phenylpyruvic (1)	Phe/Ala = 1.0	Phe/Ala = 100
10. compd **1** (0.7)	4-*t*-BuPhepyr[e] (1) phenylpyruvic (1)	4-*t*-BuPhe/Phe > 150	4-*t*-BuPhe/Phe > 150[h]
11. compd **1** (0.7)	2-M-5-BPhepyr[i] (1) pyruvic (10)	MBPhe/Ala = 0.2 ± 0.1	MBPhe[k]/Ala = 2 ± 1[h]
12. compd **1** (0.9)	2-M-5-BPhepyr[i] (1) pyruvic (10)	MBPhe/Ala = 0.13	MBPhe[k]/Ala = 1.3[f]
13. endo pair **17** (0.7)	pyruvic (100) phenylpyruvic (1)	Phe/Ala = 0.5 ± 0.2	Phe/Ala = 50 ± 20
14. endo pair **17** (0.7)	4-*t*-BuPhepyr[e] (1) phenylpyruvic (1)	4-*t*-BuPhe/Phe > 30	4-*t*-BuPhe/Phe > 30[j]
15. endo pair **17** (0.7)	4-*t*-BuPhepyr[e] (1) pyruvic (100) 4-*t*-Buphenol (5)	4-*t*-BuPhe/Ala = 0.4 ± 0.07	4-*t*-BuPhe/Ala = 41 ± 7[k]
16. endo pair **17** (0.7)	2-M-5-BPhepyr[i] (1) pyruvic (10)	MBPhe/Ala = 0.28	MBPhe[l]/Ala = 2.8[h]
17. exo pair **16** (0.7)	pyruvic (100) phenylpyruvic (1)	Phe/Ala = 0.12 ± 0.05	Phe/Ala = 12 ± 5
18. exo pair **16** (0.7)	4-*t*-BuPhepyr[e] (1) phenylpyruvic (1)	4-*t*-BuPhe/Phe > 30	4-*t*-BuPhe/Phe > 30[j]
19. exo pair **16** (0.7)	4-*t*-BuPhepyr[e] (1) pyruvic (100) 4-*t*-Buphenol (5)	4-*t*-BuPhe/Ala = 0.08 ± 0.02	4-*t*-BuPhe/Ala = 8 ± 2[k]
20. exo pair **16** (0.7)	2-M-5-BPhepyr[i] (1) pyruvic (10)	MBPhe/Ala = 4	MBPhe[l]/Ala = 40[h]
21. macrocycle **18** (0.9)	pyruvic (10) 4-*t*-BuPhepyr[e] (1)	4-*t*-BuPhe/Ala = 0.39 ± 0.06	4-*t*-BuPhe/Ala = 3.9 ± 0.6[f]
22. macrocycle **18** (0.9)	pyruvic (25) 4-*t*-BuPhepyr[e] (1)	4-*t*-BuPhe/Ala = 0.4 ± 0.08	4-*t*-BuPhe/Ala = 10 ± 2[g]
23. macrocycle **18** (0.9)	2-M-5-BPhepyr[i] (1) pyruvic (10)	MBPhe/Ala = 0.15 ± 0.08	MBPhe[l]/Ala = 1.5 ± 0.8[f]

[a] In 4 M aqueous phosphate buffer, pH 8.0, unless otherwise noted. [b] Product ratios extrapolated to zero time. [c] The relative reactivities of the corresponding ketoacids, listed here for convenience with the standard amino acid abbreviations. [d] An early value, less reliable than the ratios of ca. 4 from entries 2 and 7. [e] 4-*tert*-butylphenylpyruvic acid (**2**). [f] In 60% ethylene glycol and 40% aqueous 2.7 M pH 8.0 phosphate buffer, by volume. [g] In 40% ethylene glycol and 60% aqueous 2.7 M pH 8.0 phosphate buffer, by volume. [h] In 15% ethylene glycol and 85% aqueous 2.7 M pH 8.0 phosphate buffer, by volume. [i] 2-Methoxy-5-*tert*-butylphenylpyruvic acid (**3**). In a control reaction, this was equal in reactivity to pyruvic acid with simple pyridoxamine. [j] In 9% ethylene glycol and 91% aqueous 2.7 M pH 8.0 phosphate buffer, by volume. [k] In 30% ethylene glycol and 70% aqueous 2.7 M pH 8.0 phosphate buffer. [l] 2-Methoxy-5-*tert*-butylphenylalanine.

by HPLC after derivatization with *o*-phthaldehyde. We found that the ratio of amino acids formed in competition experiments decreased with time, as the system equilibrated. For example, Figure 1 shows the peak ratios as a function of time in a competition experiment with the simple cyclodextrin–pyridoxamine **1** between phenylpyruvic acid and 100 times as much α-ketoglutaric acid, forming phenylalanine and glutamic acid, respectively. The data were fit with a curve, and the competitive values were obtained by extrapolation to time zero. The data are listed in Table I.

In the experiment shown in Figure 1, the 100:1 ratio of α-ketoglutaric acid to phenylpyruvic acid still led to a 6.7-fold preference for the formation of phenylalanine. Thus the phenylpyruvic acid is 670 times more reactive with **1** than is α-ketoglutaric acid. As entry 8 in Table I shows, the average of several runs is a 750-fold competitive advantage for phenylpyruvic acid. Much of this is because the phenyl group can bind into the cyclodextrin cavity and the ketoglutarate residue cannot, but some also reflects intrinsic reactivity. We find that even with simple pyridoxamine (Table I, entries 1, 2, and 7) there is a 4-fold preference for reaction with phenylpyruvic acid, probably because of deactivation of the keto group in α-ketoglutaric acid by hydroxyactonization. Thus the effect due to phenyl binding is a 190-fold increase. This is also consistent with our finding, similar to that we reported earlier,[5] that phenylpyruvic acid is similar in reactivity to pyruvic acid with simple pyridoxamine (Table I, entries 3, 4, and 7) but is ca. 100 times more reactive with compound **1** (Table I, entry 9).

The addition of a *tert*-butyl group to phenylpyruvic acid has remarkable effects on these competitions. In the para position the *tert*-butyl group adds to the reactivity: 4-*tert*-butylphenylpyruvic acid (**2**) is now at least 150-fold more reactive with **1** than is phenylpyruvic acid itself (Table I, entry 10). With pyridoxamine there is essentially no difference between pyruvic acid, phenyl-

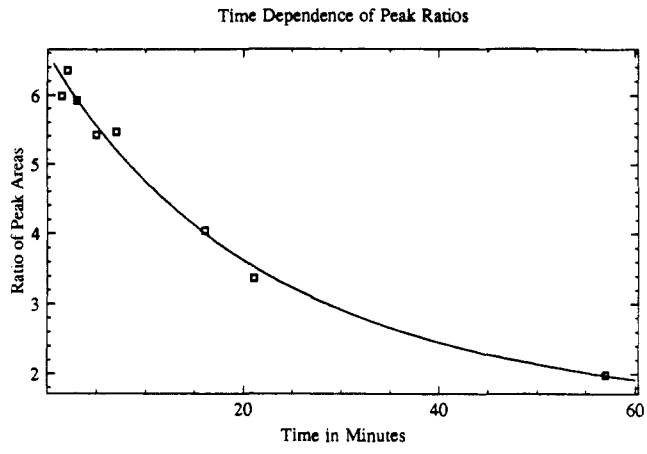


Figure 1. The observed ratio of phenylalanine to glutamic acid formed in a competitive transamination of 1 part phenylpyruvic acid and 100 parts α-ketoglutaric acid by compound **1**. The ratio decreases with time as the system equilibrates, so the kinetic competitive ratio is obtained by extrapolation to zero time.

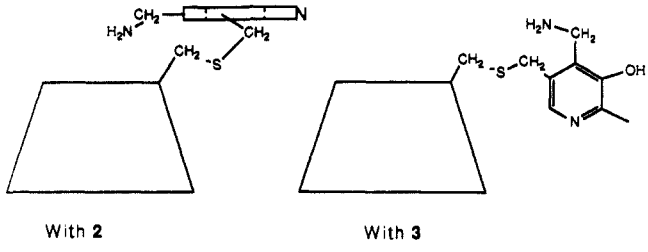


Figure 2. The geometries (from molecular models) assumed by compound **1** in the transamination of the *p-tert*-butylphenylpyruvic acid (**2**) and of the *m-tert*-butyl derivative **3**.

pyruvic acid, and *p-tert*-butylphenylpyruvic acid (Table I, entries 3–7). Thus the binding effect contributes a factor of at least 28 000-fold with **1** compared with a nonbinding substrate. Since use of the lower buffer concentration in entry 10 and the addition of some ethylene glycol both lead to a decrease in hydrophobic binding,[16] the effect in pure H_2O with 4 M buffer would be even larger.

However, a *tert*-butyl group in the meta position essentially wipes out the entire binding effect.[17] In competition experiments compound **3**, with a *m-tert*-butyl group, is at most twice as reactive with **1** as is simple pyruvic acid itself with two different solvent mixtures (Table I, entries 11 and 12).

Molecular modelling indicates that the bound *m-tert*-butylphenylpyruvic acid derivative **3** can still undergo transamination by interaction with the pyridoxamine group of **1**, but the geometry is different from that for transamination of the *p-tert*-butyl derivative **2**. In the latter case the pyridine plane can be parallel to the plane of the cyclodextrin face (Figure 2), but with the meta-substituted **3** transamination puts the pyridine ring parallel to the cyclodextrin cavity axis and perpendicular to the cyclodextrin face. Apparently the geometry in the complex with the *p-tert*-butylated substrate **2** is much preferred.

The flexibility of the linkage between the pyridoxamine and the cyclodextrin group permits adoption of the two geometries shown in Figure 2. Thus we wanted to examine derivatives in which this geometry is restricted. The rigidly linked systems **16** and **17**, and the macrocyclic derivative **18**, all have such restrictions. We hoped, and observed, that these restrictions would affect the competitive reactivities of substrates **2** and **3**, with their own restricted geometries. Additionally, rigid restriction of the transaminase mimics to the correct geometry might be expected to lead to greatly increased reactivity in the right cases.

The results were quite interesting. As the data in Table I show, the additional linkage in either the endo pair of isomers or the exo pair of isomers led to a *decrease* compared with **1** in the selectivity for transamination of phenylpyruvic acid relative to pyruvic acid (Table I, the 50-fold selectivity of entry 13 and the 12-fold selectivity of entry 17 compared with the 100-fold selectivity of entry 9). Both ratios reflect hydrophobic binding;[16] they decrease to 37-fold for entry 13 and to 6-fold for entry 17, when the buffer concentration is lowered to 2.7 M. Apparently neither geometry set by the double linkage is ideal for transamination, and the extra flexibility of the singly linked compound **1** produces an advantage.

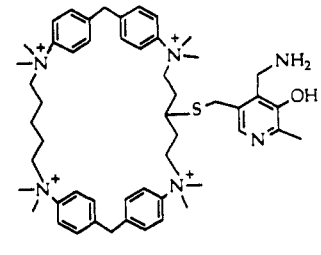
The selectivity for *p-tert*-butylphenylpyruvic acid is quite large, so it was artificially suppressed by adding 4-*tert*-butylphenol, a competitive binder, and by adding more ethylene glycol and using a lower buffer concentration. As the data in Table I show (entries 15 and 19), this led to the finding that the endo pair of compounds **17** are more effective than is the exo pair **16**.

The actual finding is that the chromatographically more polar pair of isomers gives the data of entry 15, and the less polar pair of isomers give the data of entry 19. This finding and related reasons, discussed below, leads us to assign the endo geometry to the polar pair of isomers (AB and BA), while the less polar pair is assigned the exo geometry. The pyridoxamine unit can lie directly above the cyclodextrin cavity in the endo geometry, in an excellent position to react with the axially directed ketoacid unit of substrate **2**. The exo geometry orients the pyridoxamine unit off to the side, not within easy reach of an axially directed substrate carbonyl.

The situation with the *m-tert*-butylated substrate **3** is quite different. Here the nonpolar pair of isomers, to which we assign the exo geometry, is actually more effective (Table I, entry 20) than is the polar pair of isomers (entry 16) or the singly linked flexible transaminater **1** (entries 11 and 12). In this case the restricted geometry of the doubly linked compounds gives them an advantage over the singly linked compounds. This is again reasonable if the nonpolar isomers indeed have the exo geometry; this points the pyridoxamine unit off to the side of the cavity, just as the binding *m-tert*-butyl group points the ketoacid grouping off to the side in the complex of **3**.

As a final piece of evidence for the assignments of endo geometry to the polar pair of isomers and exo geometry to the nonpolar pair, there is a difference in reactivity during hydrolysis of the acetonide groups in intermediates **12** and **13** of Scheme IV. Hydrolysis under the same conditions was observed to be faster with the nonpolar pair. Since in the exo isomers it is less likely that the cyclodextrin will shield the acetonide group from hydrolysis, this observation is in accord with our assignments.

We had described[3,8] transaminations by the singly linked pyridoxamine/macrocycle compound **25**, which showed a significant preference for phenylpyruvic acid compared with nonbinding substrates. We have now examined transaminations by **18**, which is doubly linked although not in the same position as was the single linkage of **25**.



25

The data are listed in Table I. It is apparent (entries 21 and 22) that the transaminater **18** is poorly selective for *p-tert*-bu-

(16) Cf.: Breslow, R.; Guo, T. *J. Am. Chem. Soc.* **1988**, *110*, 5613.
(17) Both *p-tert*-butylphenyl and *m-tert*-butylphenyl groups bind strongly into β-cyclodextrin in water. For example, both *m*- and *p-tert*-butylphenol have $K_{binding}$ of greater than 10^4 M^{-1}. Cf.: Nishioka, T.; Fujita, T. *Topics Curr. Chem.* **1985**, *128*, 61–89.
(18) Olah, G.; Ohannesian, L.; Arvanaghi, M. *J. Org. Chem.* **1984**, *49*, 3856.
(19) Kortnyk, W.; Wiedman, W. *J. Chem. Soc.* **1962**, *2531*.
(20) Sekine, M.; Matsuzaki, J.; Hata, T. *Tetrahedron* **1985**, *41*, 5279.
(21) Product P 0532, obtained from Sigma Chemical Co. as a solution.

tylphenylpyruvic acid relative to pyruvic acid, so binding into the cavity is not very helpful. By contrast, the previous macrocyclic pyridoxamine compound **25** was quite selective for hydrophobic substrates,[3] even more selective than the cyclodextrin compound **1**. Thus the altered geometry in our new macrocyclic derivative **18** has made all the difference. The pyridoxamine in **18** is held well to the side of the cavity; while models show that it could reach a bound 4-*tert*-butylpyruvic acid, apparently this binding of the pyridoxamine to the side of the macrocyclic cavity is not optimal for reaction. We reached the same conclusion above in the case of the exo versus endo cyclodextrin derivatives **16** and **17**.

In that case, the *m-tert*-butylphenylpyruvic acid substrate **3** was better for reaction with the exo isomer, since the side chain of the substrate projects toward the edge of the binding ring. The data in Table I (entry 23) show that this was not true in the case of the new macrocycle **18**, which showed no special reactivity toward the meta substrate isomer. In the exo cyclodextrin system **16** there is a conformation with the pyridoxamine held well away from the cavity, that in models cannot transaminate any of the substrates we have examined, and another conformation with the pyridoxamine closer. This is the conformation that we believe is able to react with the *m-tert*-butyl substrate **3**. In contrast, macrocycle **18** has its pyridoxamine unit held fairly rigidly away from the binding site. It will probably show its optimum transaminating activity toward other substrates with even larger projections away from the binding site axis.

Conclusions

1. Careful separation of kinetic effects from subsequent equilibration shows that the singly linked pyridoxamine/cyclodextrin compound **1**, a mimic for transaminase enzymes, is even more selective for binding substrates such as phenylpyruvic acid than was previously believed.

2. Additional of a *tert*-butyl group to phenylpyruvic acid greatly increases this substrate selectivity if it is in the para position, but largely erases it if it is in the meta position. Thus there is a significant optimum in the binding geometry required.

3. A novel free radical method permits us to synthesize a pyridoxamine derivative carrying an ethanedithiol unit at C-5. A new procedure has been developed for the synthesis of a cyclodextrin derivative with iodine atoms on neighboring glucose residues. These compounds have been used to synthesize four cyclodextrin derivatives with double linkages. One pair of isomers with the pyridoxamine nearer the cyclodextrin ring axis selectively transaminates the *p-tert*-butylphenylpyruvic acid substrate, while the other pair of isomers is better adapted to the meta substrate isomer. Thus here the geometries of substrate and catalyst are made complementary.

4. A novel doubly linked pyridoxamine/macrocycle compound has been made. In contrast to a previously studied analogue with a single linkage, or to the cyclodextrin examples, this one shows little cooperativity between binding and transamination selectivity for the substrates examined. Its geometry will require yet other substrates for optimal reaction.

Experimental Section

***p-tert*-Butylphenylpyruvic Acid (2).** Freshly recrystallized *N*-acetylglycine (1.3 g, 0.011 mol), *tert*-butylbenzaldehyde (3.0 g, 0.018 mol), sodium acetate (0.675 g, 0.008 mmol), and 2.7 mL of acetic anhydride were heated at 110 °C for 90 min. The solution was allowed to cool and then concentrated. The residue was taken up in 9 mL of acetone and 23 mL of water and boiled for 4 h. The acetone was evaporated, and the mixture was partitioned between 30 mL each of water and ether. The layers were separated, and the aqueous layer was further extracted with two 20-mL portions of ether. A drop of acetic acid was added to the combined ether portions, and the solution was left in the refrigerator for several hours. The solution was filtered, and the solid was washed with cold ether to afford 1.33 g (0.0051 mol, 46% yield) of *p*-(*tert*-butyl)-2-acetamidocinnamic acid as yellow crystals: mp 231–233 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 7.54 (d, 2 H), 7.48 (s, 1 H), 7.45 (d, 2 H), 2.13 (s, 3 H), 1.33 (s, 9 H); MS (CI, NH_3) 279 (20%, M + NH_3), 262 (100%, M + H^+).

This (0.1 g, 0.38 mmol) was slurried in 1 M HCl solution (15 mL) and heated under reflux for 12 h. After cooling, the solution was partitioned with 15 mL of ether, and the aqueous layer was further extracted with two 10-mL portions of ether. The combined ether layers were concentrated, and the residue was taken up in CH_2Cl_2 and filtered. The filtrate was concentrated, and the residue was dissolved in 5 mL of methanol and precipitated with 20 mL of water. After cooling, the solid **2** was collected on a fritted glass funnel (0.06 g, 0.27 mmol, 71% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.73 (d, 2 H), 7.42 (d, 2 H), 6.67 (s, 1 H), 1.33 (s, 9 H). MS (CI, CH_4), 179 (25%, M − *t*-Bu + CH_4), 163 (100%, M − *t*-Bu).

***m-tert*-Butyl-2-methoxyphenylpyruvic Acid (3).** 4-*tert*-Butylanisole (5.0 g, 30.5 mmol) was dissolved in 200 mL of dry ether. A hexane solution of *n*-butyllithium (50.0 mmol) was added, and the solution was stirred for 20 h. *N*-Formylpiperidide[18] (6.0 mL, 54.0 mmol) was added, and the solution was stirred for 30 min. Aqueous 3 N HCl (50 mL) was then added until the solution was acidic, an additional 150 mL of water was added, and the mixture was partitioned. The aqueous layer was further extracted with three 25-mL portions of ether. The combined organic layers were washed with saturated $NaHCO_3$ and then brine, dried over anhydrous Na_2SO_3, and concentrated. Chromatography on silica (5% ethyl acetate in hexane) afforded 2-methoxy-5-*tert*-butylanisole (2.81 g, 14.6 mmol, 48% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.48 (s, 1 H), 7.85 (d, J = 1 Hz, 1 H), 7.58 (dd, J = 7.6, 1 Hz, 1 H), 6.92 (d, J = 7.6 Hz, 1 H), 3.91 (s, 3 H), 1.33 (s, 9 H).

This aldehyde (1.48 g, 7.71 mmol), *N*-acetylglycine (0.903 g, 7.71 mmol), acetic anhydride (1.5 mL), and sodium acetate (0.3 g) were heated to 110 °C with stirring for 1.5 h. The acetic anhydride was then removed by evaporation. Acetone (4 mL) and water (11 mL) were added, and the mixture was heated under reflux for 10 h. The acetone was removed by evaporation, 10 mL of brine was added, and the mixture was extracted with four 20-mL portions of CH_2Cl_2. The combined organic layers were concentrated, and the residue was chromatographed on silica (10% methanol in CH_2Cl_2), affording *m-tert*-butyl-*o*-methoxy-2-acetamidocinnamic acid as a yellow solid (0.7 g, 31% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.45 (s, 1 H), 7.57 (s, 1 H), 7.41–7.36 (m, 2 H), 6.92 (d, J = 8.65 Hz, 1 H), 3.90 (s, 3 H), 2.10 (s, 3 H), 1.28 (s, 9 H); MS (EI), 291 (7%), 273 (12%), 193 (70%), 177 (53%).

This cinnamic acid derivative (0.10 g, 0.34 mmol) was slurried in 1 M HCl (30 mL) and heated to reflux for 12 h. The cooled mixture was extracted with three 15-mL portions of ether. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The product **3** (0.079 g, 0.32 mmol, 94% yield) appeared to be homogeneous by ^{1}H NMR and was used without further purification. The product is a colorless oil which must be stored in the freezer: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.29 (dd, J = 1, 8 Hz, 1 H), 7.15 (d, J = 1 Hz, 1 H), 6.93 (br s, 1 H), 6.79 (d, J = 8 Hz, 1 H), 4.18 (s, 2 H), 3.74 (s, 3 H), 1.28 (s, 9 H); MS (EI), 250 (75%), 235 (100%), 207 (75%), 177 (77%).

Pyridoxine Acetonide Aldehyde 6. The hydrochloride salt of pyridoxine acetonide **5**[19] (13.7 g, 58 mmol) was converted to the free base with concentrated ammonia. This was dissolved in $CHCl_3$ (1 L), MnO_2 (75 g, 870 mmol) was added, and the mixture was stirred for 12 h. The MnO_2 was removed by filtration through Celite, and the filtrate was concentrated. The product **6** (8.3 g, 40 mmol, 69% yield) was isolated by chromatography on silica (5% THF in CH_2Cl_2): ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.03 (s, 1 H), 8.46 (s, 1 H), 5.17 (s, 2 H), 2.50 (s, 3 H), 1.55 (s, 6 H).

Pyridoxine Acetonide Olefin 7. Methyltriphenylphosphonium bromide (17.2 g, 48.3 mmol) was dissolved in 240 mL of dry THF. The solution was cooled to 0 °C, and *n*-butyllithium (44.3 mmol, solution in hexanes) was added by syringe. The solution was allowed to warm to room temperature and to stir 1 h. It was then cooled to −78 °C, and the aldehyde **6** (8.30 g, 40.3 mmol) was added as a solution in 240 mL of THF. After 10 min, the mixture was allowed to gradually warm to room temperature. The mixture was then poured into 500 mL of saturated $NaHCO_3$. The layers were partitioned, and the aqueous layer was further extracted with three 200-mL portions of CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentated. Chromatography on silica (5–6% THF in CH_2Cl_2) provided pure **7** (8.0 g, 39 mmol, 97% yield) as an off-white foam: ^{1}H NMR (200 MHZ, $CDCl_3$) δ 8.13 (s, 1 H), 6.51 (dd, 1 H, J = 11, 17 Hz), 5.65 (d, 1 H, J = 17 Hz), 5.38 (d, 1 H, J = 11 Hz), 4.83 (s, 2 H), 2.39 (s, 3 H), 2.53 (s, 6 H).

Pyridoxine Acetonide Bis(Ethyl xanthate) (9). To a solution of the olefin **7** (0.58 g, 2.85 mmol) in 40 mL of benzene was added diethyldixanthogen **8** (2.07 g, 8.55 mmol). The solution was cooled to 0 °C and photolyzed with a sun lamp for 8 h. The benzene was removed under reduced pressure. Flash chromatography of the crude residue on silica (150 g) with 5% ethyl acetate in CH_2Cl_2 afforded 1.03 g (81% yield) of the bis(xanthate) **9**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.04 (s, 1 H), 4.97 (m, 3 H), 4.63 (q, 4 H, J = 7 Hz), 3.83 and 3.69 (dAB, 2 H, J = 9, 16

Hz), 2.38 (s, 3 H), 1.55 (s, 3 H), 1.53 (s, 3 H), 1.40 (t, 6 H, J = 7 Hz).

β-Cyclodextrin *A,B*-Diiodide (10). 4,6-Dimethoxybenzene-1,3-bis-(sulfonyl) chloride[20] (3.5 g, 10.4 mmol) was dissolved in 100 mL of dry pyridine and added over 5 min to a 200-mL solution of dry pyridine containing β-cyclodextrin (12 g, 10.5 mmol). The mixture was warmed to 40 °C for 2 h, and the pyridine was removed by evaporation under vacuum at room temperature. The residue was chromatographed by using a gradient of methanol and water (30–80%) in reverse-phase chromatography. After concentration and lyophilization, an off-white powder (2.7 g, 1.9 mmol, 12% yield) was obtained which was the pure bridged disulfonate by TLC and ^{1}H NMR: ^{1}H NMR (200 MHz, D_2O) δ 8.10 (s, 1 H), 6.83 (s, 1 H), 4.98–4.83 (m, 7 H), 4.6–4.2 (m, 4 H), 4.00 (s, 3 H), 3.98 (s, 3 H), 3.9–3.0 (m, ~31 H). This material was normally converted directly into the AB-diiodide by heating with excess KI in dry dimethylacetamide as reported by Tabushi.[14]

Acetonides 12 and 13. To the bis(xanthate) ester **9** (0.43 g, 0.97 mmol) in 25 mL of dry ether at 0 °C was added lithium aluminum hydride (0.073 g, 1.9 mmol), and the solution was stirred for 2 h. The reaction was carefully quenched with water (10 mL) and brought to pH 6 with 1.0 N H_2SO_4. The mixture was partitioned, and the aqueous layer was extracted with four 20-mL portions of dichloromethane. Exposure of the resulting product **4** to air was minimized during the course of the workup by degassing all solutions. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. Dimethylacetamide (25 mL), dry methanol (25 mL), K_2CO_3 (0.7 g) and β-cyclodextrin *A,B*-diiodide (0.16 g, 0.43 mmol) were added, and the solution heated to 55 °C for 10 h. The solvents were removed, and the residue was chromatographed on reverse-phase C_{18}-silica with a gradient from 10–80% methanol in water. Two pairs of stereoisomers were isolated: the less polar **12** (0.0635 g, 0.046 mmol, 10% yield) and the more polar **13** (0.0936 g, 0.068 mmol, 16% yield).

12: ^{1}H NMR (D_2O, 200 MHz, δ) 7.93 (s, 0.5 H), 7.88 (s, 0.5 H), 4.82–5.12 (m, 9 H), 2.28 (br s, 3 H), 1.49 (br s, 6 H); FAB-MS, 1371 (M + 1).

13: ^{1}H NMR (D_2O, 200 MHz, δ) 8.32 (s, 0.5 H), 7.88 (s, 0.5 H), 4.82–5.12 (m, 9 H), 2.28 (br s, 3 H), 1.49 (br s, 6 H). FAB-MS, 1371 (M + 1).

Pyridoxals 14 and 15 and Pyridoxamines 16 and 17. The acetone-protecting groups of **12** and **13** were removed by heating them with pyridinium tosylate for 3–12 h and then isolating the resulting pyridoxine derivatives with reverse phase chromatography. Deprotected **14**: ^{1}H NMR (D_2O, 200 MHz) δ 8.31 (s, 1 H), 4.86–5.07 (m, 9 H), 2.19 (br s, 3 H); FAB-MS, calcd for $C_{51}H_{79}O_{35}NS_2$ 1329.6, found 1331. Deprotected **15**: ^{1}H NMR (D_2O, 200 MHz) δ 7.95 (br s, 0.5 H), 7.52 (br s, 0.5 H), 4.87–5.05 (m, 9 H), 2.29 (s, 1.5 H), 2.25 (s, 1.5 H); FAB-MS, calcd for $C_{51}H_{79}O_{35}NS_2$ 1329.6, found 1331.

Each alcohol (10 mg) was dissolved in 1.5 mL of 0.6 N H_2SO_4 and stirred with 17 mg of MnO_2 for 30 min. The MnO_2 was removed by filtering through Celite/glass fibers. After neutralization with saturated bicarbonate, the solution was passed through a reverse-phase column. A yellow band formed at the top of the column, which was washed with one volume of water and one volume of 10% methanol in water. The yellow band was eluted with 30% methanol in water. Ultraviolet spectroscopy of the yellow chromatography fractions showed that they contained the air-sensitive pyridoxal derivatives **14** and **15**.

The yellow fractions were combined and concentrated at room temperature. The residue was taken up in 1.5–2.0 mL of 50% methanol/water and heated under reflux with 0.016 g of phenylglycine for 3 h. After cooling, the now nearly colorless solution was filtered and purified by SP-25 ion exchange chromatography, with a 0–1 M pyridinium acetate (pH = 5) buffer. About 2–3 mg of product amine is isolated.

For the exo isomeric mixture **16**: ^{1}H NMR (200 MHz, D_2O) δ 7.46 (br s, 0.5 H), 7.40 (br s, 0.5 H), 5.1–4.6 (m, 7 H), 4.2–2.8 (m, 47 H), 2.22 (s, 1.5 H), 2.17 (s, 1.5 H); FAB-MS, calcd for $C_{51}H_{80}O_{34}N_2S_2$ 1329, observed 1330 (65%, M + 1), 1352 (100%, M + Na).

For the endo isomeric mixture **17**: ^{1}H NMR (200 MHz, D_2O) δ 7.50 (br s, 0.5 H), 7.27 (br s, 0.5 H), 5.1–4.6 (m, 7 H), 4.2–2.8 (m, 47 H), 2.19 (s, 1.5 H), 2.15 (s, 1.5 H); FAB-MS calcd for $C_{51}H_{80}O_{34}N_2S_2$ 1329, observed 1330.6 (85%, M + 1), 1352.6 (100%, M + Na), 1368.6 (45%, M + K).

Macrocyclic Tetratosyl Ketone 19. 4,4′-Bis(tosylamido)benzophenone (25.0 g, 48 mmol) was dissolved in 125 mL of DMF, and K_2CO_3 (51 g, 381 mmol) and 1,6-dibromohexane (100 g, 410 mmol) were added. This mixture was allowed to stir at room temperature for 24 h, after which time it was poured into 800 mL of 1:1 EtOAc/H_2O. The aqueous layer was washed with ether, and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. Chromatography on silica gel (petroleum ether followed by CH_2Cl_2) afforded 24.5 g (60% yield) of the bis-*N*-bromohexylated ketone as a yellow solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.78 (d, J = 8.6 Hz, 4 H), 7.48 (d, J = 8.4 Hz, 4 H), 7.27 (d, J = 8.6 Hz, 4 H), 7.22 (d, J = 8.4 Hz, 4 H), 3.58 (broad t, J = 6.5 Hz, 4 H), 3.38 (t, J = 6.8 Hz, 4 H), 2.43 (s, 6 H), 1.81 (br t, J = 6.8 Hz, 4 H), 1.39 (m, 12 H).

A solution of this dibromide (13.4 g, 15.8 mmol) and 4,4′-bis[(tosylamido)phenyl]methane (8.0 g, 15.8 mmol) in 200 mL of DMF was added gradually over a period of 2 h to a suspension of K_2CO_3 (11.8 g, 85 mmol) in DMF which had been preequilibrated to 125 °C. The reaction mixture was heated, stirred overnight, then cooled, and poured into 800 mL of 1:1 EtOAc/water. The aqueous layer was extracted with EtOAc, and the combined organics were dried with Na_2SO_4 and concentrated on a rotary evaporator. The resulting orange solid was recrystallized from 1:1 EtOAc/EtOH to yield 7.85 g (42% yield) of the macrocycle **19**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.67 (d, J = 8.6 Hz, 4 H), 7.39 (d, J = 8.2 Hz, 8 H), 7.27 (d, J = 8.4 Hz, 8 H), 7.23 (d, J = 8.2 Hz, 4 H), 7.10 (d, J = 8.6 Hz, 4 H), 7.03 (d, J = 8.2 Hz, 4 H), 6.85 (d, J = 8.4 Hz, 4 H), 3.92 (s, 2 H), 3.50 (br t, 4 H), 3.40 (br t, 4 H), 2.38 (s, 12 H), 1.24 (m, 16 H); FAB MS, 1192 (calcd 1192); IR (KBr) 3050, 2950, 2880, 1670, 1600, 1515, 1355, 1172, 1100, 825, 720 cm^{-1}.

Tetrakis(trifluoroacetyl) Ketone 20. A solution of the above macrocyclic tetrakis(tosylamide) (67 mg, 56 mmol) in 5 mL of 2:3 H_2SO_4/HOAc was stirred for 20 h at 80 °C, then poured into 100 mL of water, and brought to pH 9 with NaOH. The solution was extracted with CH_2Cl_2, and the organics were dried over Na_2CO_3. The solvent was removed under reduced pressure to give 30 mg (93% yield) of the detosylated tetraamine as a dark green solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.66 (d, J = 8.6 Hz, 4 H), 6.97 (d, J = 8.2 Hz, 8 H), 6.56–6.50 (dd, J = 8.4, 8.8 Hz, 8 H), 4.14 (br s, 4 H), 3.75 (s, 2 H), 3.19 (t, J = 7.0 Hz, 4 H), 3.09 (t, J = 6.8 Hz, 4 H), 1.43 (br s, 8 H); MS (CI); 575 (M + 1).

To a solution of this macrocyclic tetraamine (100 mg, 174 mmol) in 5 mL of anhydrous CH_2Cl_2 was added 0.123 mL of trifluoroacetic anhydride (871 mmol, 5 equiv). The mixture was stirred at room temperature for 4 hours and then poured into saturated aqueous $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 and the combined organics were dried over Na_2SO_4. Evaporation of solvent afforded 167 mg (quantitative yield) of **20** as a yellow solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.85 (d, J = 8.2 Hz, 4 H), 7.33 (d, J = 8.0 Hz, 8 H), 7.21 (d, J = 8.2 Hz, 8 H), 7.09 (d, J = 8.0 Hz, 4 H), 4.03 (s, 2 H), 3.77 (br t, J = 6.8 Hz, 4 H), 3.65 (br t, J = 7.2 Hz, 4 H), 1.51 (s, 8 H), 1.29 (br s, 8 H).

Macrocyclic Trifluoroacetamide Pyridoxine Acetonide 21. A solution of pyridoxine acetonide dixanthate **9** (300 mg, 693 mmol) in 50 mL of dry ether was cooled in an ice bath and bubbled with Ar for 5 min, and then 200 mg (7.9 mmol) of $LiAlH_4$ was added in one portion. The ice bath was removed, and the mixture was allowed to stir and warm to room temperature over 2.5 h. At the end of this time the flask was reimmersed in an ice bath, and 100 mL of water was added carefully (dropwise at first). Then 1 N H_2SO_4 was added until pH 5.5, at which time the water layer became clear and the ether layer became slightly colored. The aqueous layer was washed with CH_2Cl_2, the combined organics were dried over Na_2SO_4, and the solvent was removed under reduced pressure. High vacuum was applied to remove the last traces of ether, then the remaining compound **4**, as a yellow oil, was taken up in 10 mL of distilled CH_2Cl_2.

To a solution of macrocyclic trifluoroacetamide **20** (250 mg, 261 mmol) in 15 mL of distilled Ar purged CH_2Cl_2 was added 1.5 mL of 1 M $TiCl_4$ in CH_2Cl_2. This solution was allowed to stir for 5 min (a yellow precipitate formed), and then the previously prepared solution of the dithiol **4** in CH_2Cl_2 was added dropwise. The mixture was allowed to stir at room temperature for 48 h, and then it was poured into a stirred mixture of 200 mL of CH_2Cl_2 and 200 mL of saturated aqueous $NaHCO_3$. The organic layer was separated and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The greenish residue was chromatographed on silica by using 2% CH_3OH in CH_2Cl_2 to yield 205 mg of the macrocyclic trifluoroacetamide pyridoxine acetonide **21**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.30 (s, 1 H), 7.74 (d, J = 8.6 Hz, 2 H), 7.60 (d, J = 8.4 Hz, 2 H), 7.23 (d, J = 8.2 Hz, 4 H), 7.12 (d, J = 8.4 Hz, 8 H), 4.76 (s + m, 3 H), 4.04 (s, 2 H), 3.71–3.55 (m, 10 H), 2.40 (s, 3 H), 1.86 (br s, 8 H + 4 H), 1.30 (br s, 8 H); FAB MS, 1212 (calcd 1211).

Macrocyclic (Dimethylamino)amide Pyridoxine Acetonide 22. To a solution of the macrocyclic trifluoroacetamide pyridoxine acetonide **21** (205 mg, 176 mmol) in 20 mL of 1:1 THF/MeOH was added 6 mL of 1 M aqueous KOH. The solution turned orange immediately and was allowed to stir for 2 h, at which time it was extracted with CH_2Cl_2. The CH_2Cl_2 extracts were dried over Na_2SO_4, and the solvent was removed under reduced pressure to yield 148 mg (quantitative yield) of the acetonide from which the trifluoroacetyl group had been removed. This tetraamine was used without purification in the following step.

^{1}H NMR (200 MHz, $CDCl_3$) δ 8.30 (s, 1 H), 7.47 (d, J = 8.6 Hz, 2 H), 7.35 (d, J = 8.8 Hz, 2 H), 6.94 (d, J = 7.0 Hz, 4 H), 6.50 (m, 8

H), 4.85 (s, 2 H), 4.70 (m, 1 H), 3.74 (s, 2 H), 3.50 (m, 2 H), 3.07 (m, 8 H), 2.38 (s, 3 H), 1.52 (br s, 8 H + 4 H), 1.41 (br s, 8 H).

To a solution of the macrocyclic tetraamine pyridoxine acetonide (148 mg, 179 mmol) in 25 mL of CH_2Cl_2 was added 15 mL of saturated aqueous $NaHCO_3$. The two-phase mixture was stirred vigorously, and 350 mL of chloroacetyl chloride was added dropwise by syringe. The mixture was allowed to stir for 5 h, then the organic layer was separated and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was chromatographed on silica, eluting with 7% NH_3 saturated MeOH in CH_2Cl_2, to yield 141 mg (75%) of the macrocyclic (diethylamino)amide pyridoxine acetonide **22**.

^1H NMR (200 MHz, $CDCl_3$) δ 8.25 (s, 1 H), 7.67 (d, J = 8.4 Hz, 2 H), 7.56 (d, J = 8.4 Hz, 2 H), 7.18 (d, J = 8.2 Hz, 4 H), 7.05 ($\sim$t, $J \sim$ 8 Hz, 8 H), 4.79 (s, 2 H), 4.77 (m, 1 H), 3.99 (s, 2 H), 3.60 (m, 10 H), 2.80 (br s, 4 H), 2.74 (s, 4 H), 2.35 (s, 3 H), 2.19 (s, 24 H), 1.50 (s, 6 H), 1.41 (br s, 8 H), 1.22 (br s, 8 H); FAB MS, 1169 (calcd 1168).

Macrocyclic (Dimethylamino)amide Pyridoxine Diol **23**. To a solution of the above acetonide **22** (141 mg, 125 mmol) in 5 mL of THF was added 7 mL of 3.5 N HCl. The solution was stirred at room temperature for 17 h. The solvent was removed under reduced pressure, and the gummy residue was dissolved in 1:4 NH_3 saturated MeOH/CH_2Cl_2 and filtered to remove the fine white precipitate of inorganic salts. The filtrate was chromatographed on silica eluting with 1:4 NH_3 saturated MeOH/CH_2Cl_2 to yield 100 mg (74%) of the macrocyclic pyridoxine derivative **23**.

^1H NMR (200 MHz, $CDCl_3$) δ 8.15 (s, 1 H), 7.73 (d, J = 8.2 Hz, 2 H), 7.58 (d, J = 8.2 Hz, 2 H), 7.18 (d, J = 8.1 Hz, 4 H), 7.04 (m, 8 H), 4.84 (br s, 3 H), 3.99 (s, 2 H), 3.58 (m, 10 H), 2.96 (br s, 2 H), 2.84 (br s, 2 H), 2.75 (s, 4 H), 2.37 (s, 3 H), 2.31–2.16 (m, 24 H), 1.41 (br s, 8 H), 1.22 (br s, 8 H); FAB MS, 1129 (calcd 1128).

Macrocyclic (Dimethylamino)amide Pyridoxal Imine 24. To a solution of the above pyridoxine derivative **23** (10 mg, 9 mmol) in 5 mL of 1:1 benzene/pyridine was added 10 mg of MnO_2, 15 mL of 3-(dimethylamino)propylamine, and a few 4Å activated sieves. The solution was heated under reflux for 45 min and then centrifuged, and the yellow supernatant was decanted from the MnO_2. The solvent was removed under reduced pressure. The material is sufficiently pure at this point to be used in a subsequent reaction but can be purified by chromatography on alumina, eluting with 5% MeOH in CH_2Cl_2, to yield 5 mg (50%) of the imine **24**.

^1H NMR (200 MHz, $CDCl_3$) δ 8.20 (br s, 1 H), 7.71 (br d, J = 8 Hz, 2 H), 7.59 (br d, J = 8 Hz, 2 H), 7.22 (d, J = 8.1 Hz, 4 H), 7.05 (m, 8 H), 4.03 (s, 2 H), 3.61 (m, 10 H), 2.95 (br s, 4 H), 2.77 (s, 4 H), 2.51 (s, 3 H), 2.30–2.20 (7, 24 H), 1.45 (br s, 8 H), 1.24 (br s, 8 H); FAB MS, 1211 (calcd 1210).

This compound could be converted in the standard way to the corresponding oxime.

^1H NMR (200 MHz, $CDCl_3$) δ 8.34 (s, 1 H), 8.13 (s, 1 H), 7.80 (d, J = 8 Hz, 2 H), 7.55 (d, J = 8 Hz, 2 H), 7.3–7.0 (m, 12 H), 4.04 (s, 2 H), 3.61 (m, 10 H), 3.05 (br s, 4 H), 2.85 (s, 4 H), 2.51 (s, 3 H), 2.4 (m, 24 H), 2.0–1.0 (m, 16 H); FAB MS, 1142 (calcd 1141).

Macrocyclic (Dimethylamino)amide Pyridoxamine 18. Anhydrous NH_4OAc in MeOH was prepared by bubbling NH_3 into MeOH until saturated and then carefully adding AcOH to the cooled solution until pH 9 is reached. A 20-mg sample of the diol **23** was oxidized to the imine **24** as described previously. To the crude yellow residue from this reaction was added 25 mL of the anhydrous NH_4OAc solution. The resulting dark yellow solution was alllowed to stir for 1 h, at the end of which time 10 mg of $NaBH_4$ was added in a single portion. The yellow color of the solution vanished immediately upon this addition, and the resulting solution was allowed to stir for 3 h and then worked up by partitioning between methylene chloride and water. The 11 mg of crude material was chromatographed on silica, eluting with 20% ammonia saturated MeOH in CH_2Cl_2, to yield 4 mg of purified macrocyclic pyridoxamine **18**.

^1H NMR (200 MHz, $CDCl_3$) δ 8.15 (br s, 1 H), 7.70 (br d, 2 H), 7.6 (br d, 2 H), 7.3–7.0 (m, 12 H), 4.02 (s, 2 H), 3.58 (br s, 10 H), 3.05 (br s, 4 H), 2.8 (br s, 8 H), 2.22 (br s, 24 H), 1.5–1.1 (m, 16 H).

In order to get a FAB mass spectrum it was necessary to derivatize **18** in the following manner: to a 0.5-mg sample of the crude material was added 0.3 mL of distilled CH_2Cl_2 and a drop of acetic or of trifluoroacetic anhydride. After 3 h all of the volatiles were removed under vacuum, and the residue was submitted for a mass spectrum.

FAB MS, after acetylation, 1170 (calcd 1168 for monoacetylation) and 1212 (calcd 1210 for diacetylation); after trifluoroacetylation 1224 (calcd 1222 for monotrifluoroacetylation).

Competitive Transaminations. The two ketoacids were incubated at 22 °C with the pyridoxamine derivative in 4 M aqueous phosphate buffer, or in the media specified in Table I, at the concentrations listed in Table I. Then 25 μL of reaction solution was vortexed with 25 μL of commercial *o*-phthaldehyde/mercaptoethanol reagent solution[21] for 60 s, and the solution was allowed to incubate at ambient temperature for an additional 60 s. The amino acid derivatives were analyzed by HPLC with a C_{18} column with a programmed gradient elution profile (35/5/5 to 20/20/10, 0.1 N NaOAc/acetonitrile/methanol). When a sequence of readings was done over time, aliquots were stored at −78 °C. The peak areas were calibrated with authentic mixtures of the appropriate amino acids.

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